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Pathogenesis Traits and Antimicrobial Resistance Pattern in *Escherichia coli* Isolates Recovered From Sewage

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Abstract

Objectives: *Escherichia coli* is one of the most potential agents of community and hospital-acquired infections, which can readily acquire non-susceptibility to drugs administered to animals and humans. *E. coli*, which has been broadly applied as an indicator of fecal contamination in aquatic environments, is routinely conducted non-virulence. Nevertheless, some isolates can be virulence. The main objectives of this work were to survey antibiotic non-susceptibility and to characterize virulence factors and antibiotic resistance genes including *traT*, *fimH*, *blaCTX*, and *tetA* among the *E. coli* isolates recovered from sewage in Gonabad, in the northeast of Iran.

Materials and Methods: In this cross-sectional study, a total of 99 non-duplicate strains of *E. coli* was removed from three types of sewage including poultry (33 isolates), urban (33), and livestock slaughterhouse (33) sewages in Gonabad from May 2016 to April 2017. Then, the antimicrobial susceptibility test and extended-spectrum-beta-lactamase (ESBL) production were done based on CLSI guidelines, followed by performing the polymerase chain reaction technique to identify 2 virulence (*traT* and *fimH*) and 2 antibiotic resistance (*blaCTX* and *tetA*) genes.

Results: Meropenem was the most effective drug against the strains. The analysis of multiple drug non-susceptibility profiles in the strains showed that 39 (39.3%) strains were T_R-NA_R and three (3.03%) of them were resistant to colistin. Totally, 21 (21.2%) strains were ESBL-positive and 15 (71.42%) $ESBL^+$ isolates carried *blaCTX* gene whereas 87.9% and 100% of the strains in the livestock slaughterhouse and urban sewages carried the *tetA* gene, respectively. Finally, 85.7% of ESBL-positive isolates carried the *fimH* gene. Overall, 19.8% of ESBL-positive strains carried *fimH* and *traT* virulence genes ($fimH^+$-$traT^+$ genotype).

Conclusions: Based on the findings of this study, wastewater and surface waters are considered as significant sources of *E. coli* carrying ESBL-genes, high levels of antibiotic non-susceptibility, and pathogenesis traits. Eventually, the identified colistin non-susceptible strains are quite worrying.

Keywords: *Escherichia coli*, Sewage, Virulence, Antimicrobial resistance

Introduction

Escherichia coli, a Gram-negative bacillus in the Enterobacteriaceae family, is commonly considered as a normal flora resident of the gastrointestinal tract of animals and humans. As a result, it is one of the most frequently applied indicator organisms for contamination with feces in environments (1,2). Nevertheless, *E. coli* is also one of the most common agents of community and hospital-acquired infections which can quickly acquire non-susceptibility to the applied antibiotics in animals and humans. In addition, it consists of non-susceptibility created by extended-spectrum β-lactamases (ESBLs). The genes for ESBLs are most often placed on plasmids, which can easily be transported between the microorganisms (2).

Some strains of *E. coli* can be virulence for humans and animals (3). Further, different pathogenesis features are demonstrated for *E. coli*, which can be classified into 78 pathogenesis traits and include actions such as toxins, secretion systems, adhesins, invasions, capsules, and iron uptake systems (3-5).

Furthermore, *E. coli* is the biological indicator of water treatment safety (6). Moreover, its isolates can create various extra-intestinal and intestinal infections, including septicemia, neonatal meningitis, urinary system diseases, and diarrhea (7). Surface water is a potential source for drinking water production and is applied for the irrigating and recreating the crops, and finally, providing the contact of animals and humans to ESBL-producing *E. coli*. Exposure with these variants can straightly result in hard-to-treat infection and healthy persons (2,8).

On a global scale, drinking water contaminated with virulence organisms causes the most important health risk to humans and thither there are innumerable numbers of infection outbreaks and poisonings resulting from

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contact to poorly treated or untreated drinking water (9). Additionally, fecal pollution is an important source of virulence microbes including *E. coli* in wastewater. Accordingly, wastewater treatment protocols are planned to decrease the contaminant concentration (e.g., bacteria) in the sewage before discharge to obtain water bodies (3,10,11). As a result, non-disinfected sewages could yet possess high ratios of virulence organisms, therefore, presenting a threat to public health (3). The regions mostly affected by urban discharges harbor higher ratios of strains with characterized pathotypes (3). Thus, a potential portion of environmentally recovered *E. coli* isolates is significantly virulence (11).

Nowadays, industrial effluent is the most important source of water contamination and rises yearly because manufactures are rising as most countries are arriving industrialization (12). Industries create sewages which are queer in terms of frequency, type, and volume relating to industry type and population that applies the product. Water and wastewater management forms a practical obstacle for the beverage and food industry (13). The bacteriophages in the fecal sewage of the animal can be considered as environmental vectors for the horizontal transfer of antibiotic non-susceptibility markers (14).

Hospital wastewater is a very selective habitat and contributes to the high frequency of non-susceptible microorganisms that are poured in the natural habitats (14,15). In addition, drugs apply a selection in favor of non-susceptible organisms by suppressing susceptible bacteria growth or killing them. Further, non-susceptible organisms can adjust to environmental situations and serve as vectors for the dissemination of drug non-susceptibility (16). The potential risk for public health is that resistance markers are transported from environmental microorganisms to human pathogenic bacteria (14).

The main aims of the current research were to recognize antibiotic non-susceptibility patterns and to identify virulence factors and resistance genes including *traT* (outer membrane protein *traT* encoding gene), *fimH* (fimbriae H encoding gene), *blaCTX* (beta-lactamase resistance encoding gene), and *tetA* (tetracycline resistance encoding gene) among the *E. coli* isolates recovered from the sewage in Gonabad, located in the northeast of Iran.

Materials and Methods

Specimen Collection

In the present cross-sectional study, 99 non-duplicate strains of *E. coli* were isolated from three types of sewage, including poultry (33 isolates), urban (33), and livestock slaughterhouse (33) sewages in Gonabad from May 2016 to April 2017.

Bacterial Identification

Briefly, sewage specimens (250 mL) were collected aseptically in sterile glass bottles by directly dipping the bottles into the surface of the sewage. The samples were then transported to a microbiology laboratory for 1 hour after collection. Next, the collected samples were cultured on MacConkey agar and placed at 35°C ± 2 for 24 hours. Furthermore, the early characterization of the isolates was done by microbiological diagnostic methods. Moreover, the recovered isolates were finally distinguished by indole (+), citrate utilization (-), triple sugar iron agar inoculation (Acid/Acid), catalase (+), motility (+), H2S production (-), and oxidase (-). In this research, prototype strain *E. coli* ATCC 25922 was used as quality control (7).

Antimicrobial Susceptibility Testing

The antibiotic sensitivity test was performed by the disk diffusion (DD) technique based on CLSI recommendations (17). Different antibiotic disks (Mast Co. Ltd, UK) were applied for the test such as cefepime (CPM, 30 µg), aztreonam (ATM, 30 µg), ceftazidime (CAZ, 30 µg), ampicillin (AP, 10 µg), imipenem (IMI, 10 µg), ertapenem (ETP, 10 µg), meropenem (MEM, 10 µg), ciprofloxacin (CIP, 5 µg), ceftriaxone (CRO, 30 µg), doripenem (DOR, 10 µg), cefoxitin (FOX, 30 µg), chloramphenicol (C, 30 µg), piperacillin (PRL, 100 µg), ampicillin-sulbactam (SAM, 20 µg), amoxicillin-clavulanate (AUG, 30 µg), colistin sulphate (CO, 10 µg), piperacillin/tazobactam (PTZ, 100 + 10 µg), cefotaxime (CTX, 30 µg), gentamicin (GM, 10 µg), amikacin (AK, 30 µg), tetracycline (T, 30 µg), cefuroxime (CXM, 30 µg), norfloxacin (NOR, 10 µg), ofloxacin (OFX, 5 µg), ceftazidime (CAZ, 30 µg), nalidixic acid (NA, 30 µg), trimethoprim-sulfamethoxazole (TS, 25 + 23.75 µg), tigecycline (TGC, 15 µg), trimethoprim (TM, 5 µg), and nitrofurantoin (NI, 300 µg). *E. coli* ATCC 25922 was applied as quality control.

Detection of Multidrug-resistant Strains

Multidrug-resistant (MDR) *E. coli* isolates were described as non-susceptible to at least three antimicrobials belonging to several categories of drugs, including gentamicin, cefuroxime, and ciprofloxacin according to disk diffusion results. The data were analyzed by considering CLSI recommendations (17).

Characterization of Carbapenemase Producer Strains

The production of carbapenemase among the strains was identified by the modified Hodge test (MHT) phenotypic method based on the CLSI platform (17). The MHT was carried out for carbapenemase producer strains, followed by preparing a 0.5 McFarland dilution of the *E. coli* ATCC 25922 in 5 mm of the normal saline. Additionally, a 10 µg ertapenem antibiotic susceptibility disk was located in the center of the MHA plate. Then, the test strain was streaked in a straight line from the edge of the disk to the edge of the plate and the MHA plate was incubated in ambient air at 35 ± 2°C for 16 to 24 hours. After 24 hours of incubation, the MHT positive test revealed a clover leaf-like indentation of the *E. coli* 25922 growing along the test strain growth streak within the disk diffusion zone.

Finally, the MHT negative test showed no growth of the *E. coli* 25922 along the test strain growth streak within the disk diffusion.

Identification of ESBL Producer Strains

Combination Disc Diffusion Method

All strains were analyzed for ESBL production based on CLSI guidelines using the confirmatory disk diffusion technique (17). A ceftazidime (30 µg) and ceftazidime + clavulanic acid (30 µg + 10 µg), cefotaxime (30 µg) and cefotaxime + clavulanic acid (30 µg + 10 µg) discs (Mast, UK) were located at a distance of >24 mm on a Mueller-Hinton Agar plate, inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and placed overnight at 35 ± 2°C. A ≥5 mm rise in the inhibition zone diameter for the combination disc versus cefotaxime or ceftazidime discs confirmed ESBL production.

Detection of Virulence and Resistance Genes by Polymerase Chain Reaction

The polymerase chain reaction technique was done for the characterization of 2 virulence markers (*traT* and *fimH*) and 2 antibiotic resistance genes (*blaCTX* and *tetA*) among 99 *E. coli* strains by applying primer pairs listed in Table 1. All bacterial isolates were examined, which were finally identified and confirmed as *E. coli* by biochemical and morphological tests. The recovered strains were cultured overnight on MHA and the DNA was extracted from single recovered colonies by the boiling technique and applied as a template for the PCR assay (18-20). The sequences of the primers and the expected sizes of the amplicons are shown in Table 1. The PCR mixture consisted of 2.5 µL of the PCR buffer, 2.5 units of Taq DNA polymerase (Thermo Scientific), 10 pmol of each primer, 1 µL of dNTP mix (Thermo Scientific Company), and 2 µL of DNA template and molecular grade distilled water was used to adjust the reaction volume to 25 µL. In addition, DNA amplification was conducted in the temperature gradient thermal cycler (Eppendorf 96-well, Germany). Further, the PCR program procedure (18-20) included pre-denaturation at 95°C for 5 minutes (one cycle), 35 cycles (denaturation at 95°C for 30 seconds, annealing at the optimal temperature for each gene for 30 seconds (Table 1), and extension at 72°C for 30 seconds), followed by one cycle of final extension at 72 °C for 5 minutes. Similarly, 5 µL of PCR products was subjected to gel electrophoresis using 1.5% agarose gel (Merck, Germany), prepared in the Tris-Borate-EDTA buffer for 1 hour, and then stained with UV illuminating dye (Gel Red) and visualized by the UV-gel documentation system (Kodak Gel Logic 200, USA). Eventually, the DNA ladder (size range 50 bp) was used to detect the size of the expected bands.

Statistical Analysis

The statistical interpretation of the findings was carried out by SPSS 16 and P values ≤ 0.05 were considered statistically significant. In addition, standard deviations and means were calculated as required for numerical variables.

Results

A total of 99 *E. coli* strains was investigated in the current study. All strains were confirmed by biochemical assays. Further, the isolates were recovered from urban (33.3%, n = 33), poultry (33.3%, n = 33), and livestock slaughterhouse (33.3%, n = 33) sewages. Among the total isolates of bacteria, 6 (61.6%), 66 (66.7%), and 27 (27.3%) samples were isolated in autumn, winter, and spring, respectively.

As mentioned above, 99 strains were studied for susceptibility to 29 drugs based on the CLSI protocol. Tables 2 and 3 reveal the antibiotic susceptibility patterns of tested isolates. With regard to susceptibility testing results, meropenem was the most efficient drug against the strains (100% of the strains were sensitive) while tigecycline was the least effective antibiotic (83.9% of the strains were non-susceptible). Susceptibility rates for cefuroxime, gentamicin, nalidixic acid, and ciprofloxacin were 32.3%, 96%, 53.5%, and 75.8%, respectively, and the susceptibility frequency for colistin, cefepime, chloramphenicol, ceftriaxone, and co-trimoxazole was 97%, 91.9%, 68.7%, 92.9%, and 47.5%, respectively (Tables 2 and 3). Overall, the non-susceptibility rate among the isolates recovered from poultry sewage was more compared to the other strains. Among quinolones, the highest incidence of bacterial non-susceptibility was related to ciprofloxacin

Table 1. Primers Nucleotide Sequences and Conditions Applied to Amplify Virulence Determinants and Antibiotic Resistance Genes in *Escherichia coli* Strains

Amplicon Size (pb)	Annealing Temperature (°C)	Length (bp)	Sequence (5' to 3')	Primer Name	Target Gene	Virulence Marker	References
290	60	21	GGTGTGGTGCGATGAGCACAG	traT-F	*traT*	*traT*	(18)
		21	CACGGTTCAGCCATCCCTGAG	traT-R			
207	60	20	CATTCGCCTGTAAAACCGCC	fimH-F	*fimH*	*fimH*	(18)
		20	ATAACACGCCGCCATAAGCC	fimH-R			
544	55	23	TTTGCGATGTGCAGTACCAGTAA	blaCTX-F	*blaCTX*	*blaCTX*	(19)
		22	CGATATCGTTGGTGGTGCCATA	blaCTX-R			
494	60	20	TTGGCATTCTGCATTCACTC	tetA-F	*tetA*	*tetA*	(20)
		20	GTATAGCTTGCCGGAAGTCG	tetA-R			

Table 2. Susceptibility Pattern of Studied *Escherichia coli* Isolates to the Beta-lactam Antibiotics According to Specimen Type

Source	Betalactam Class															
	Carbapenems				Monobactam	Penicillins					Cephalosprins					
	IMI	MEM	ETP	DOR	ATM	AP	PRL	PTZ	SAM	AUG	CTX	CAZ	CXM	CRO	CPM	FOX
Poultry sewage (n=33)	32 (97)	33 (100)	33 (100)	33 (100)	32 (97)	26 (78.8)	27 (81.8)	30 (90.9)	29 (87.9)	33 (100)	31 (93.9)	32 (97)	15 (45.5)	33 (100)	32 (97)	33 (100)
Livestock slaughterhouse sewage (n=33)	33 (100)	33 (100)	33 (100)	32 (97)	31 (93.9)	29 (87.9)	28 (84.8)	33 (100)	31 (93.9)	32 (97)	31 (93.9)	33 (100)	7 (21.2)	31 (93.9)	31 (93.9)	33 (100)
Urban sewage (n=33	33 (100)	33 (100)	32 (97)	33 (100)	27 (81.8)	22 (66.7)	22 (66.7)	31 (93.9)	28 (84.8)	29 (87.9)	31 (93.9)	33 (100)	10 (30.3)	28 (84.8)	28 (84.8)	32 (97)
Total (n=99)	98 (99)	99 (100)	98 (99)	98 (99)	90 (90.9)	77 (77.8)	77 (77.8)	94 (94.9)	88 (88.9)	94 (94.9)	89 (89.9)	93 (93.9)	32 (32.3)	92 (92.9)	91 (91.9)	98 (99)

Note. IMI: Imipenem; MEM: Meropenem; ETP: Ertapenem; DOR: Doripenem; ATM: Aztreonam; AP: Ampicillin; PRL: Piperacilin; PTZ: Piperacillin/tazobactam; SAM: Ampicillin-sulbactam; AUG: Amoxicillin-clavulanate; CTX: Cefotaxime; CAZ: Ceftazidime; CXM: Cefuroxime; CRO: Ceftriaxone; CPM: Cefepime; FOX: Cefoxitin.

Table 3. Susceptibility Pattern of Studied *Escherichia coli* Isolates to the Non-betalactam Antibiotics According to Specimen Type

Source	Antibiotic Classes												
	Quinolones			Aminoglycosides		Tetracycline		Other					
	CIP	OFX	NOR	GM	AK	T	TGC	C	TS	CO	TM	NA	NI
Poultry sewage (n=33)	15 (45.5)	19 (57.6)	19 (57.6)	30 (90.9)	32 (97)	8 (24.2)	6 (18.2)	13 (39.4)	5 (15.2)	33 (100)	7 (21.2)	4 (12.1)	31 (39.9)
Livestock slaughterhouse sewage (n=33)	32 (97)	32 (97)	33 (100)	33 (100)	25 (75.8)	20 (60.6)	2 (6.1)	31 (93.9)	27 (81.8)	32 (97)	31 (93.9)	27 (81.8)	32 (97)
Urban sewage (n=33)	28 (84.8)	31 (93.9)	30 (90.9)	32 (97)	30 (90.9)	2 (6.1)	8 (24.2)	24 (72.7)	15 (45.5)	31 (93.9)	19 (57.6)	22 (66.7)	33 (100)
Total (n=99)	75 (75.8)	82 (82.8)	82 (82.8)	95 (96)	87 (87.9)	44 (44.4)	16 (16.2)	68 (68.7)	47 (47.5)	96 (97)	57 (57.6)	53 (53.5)	96 (97)

Note. CIP: Ciprofloxacin; OFX: Ofloxacin; NOR: Norfloxacin; GM: Gentamicin; AK: Amikacin, T: Tetracycline; TGC: Tigecycline; C: Chloramphenicol; TS: Trimethoprim-sulfamethoxazole; CO: Colistin sulphate; TM: Trimethoprim; NA: Nalidixic acid; NI: nitrofurantoin.

(75.8%, n = 75), followed by norfloxacin (82.8%, n = 82) and ofloxacin (82.8%, n = 82).

The analysis of multiple antibiotic resistance patterns in the isolates indicated that 39 (39.3%) strains were T_R-NA_R, followed by CXM_R-T_R (36 isolates) and T_R-NA_R-AP_R (13 strains). The interpretation of cross-resistance data showed that more than 3.03% of the strains were non-susceptible to cefuroxime, tetracycline, and gentamicin, and 3 (3.03%) of them were non-susceptible to augmentin and nalidixic acid. Furthermore, 12 (12.1%) surveyed strains were non-susceptible to ampicillin, tigecycline, and nalidixic acid.

The MHT positive isolate was not identified based on the antibiotic susceptibility test. Only 3 (3.03%) strains were resistant to colistin. Totally, 21 (21.2%) strains were ESBL-positive and 15 (71.42%) ESBL-positive isolates carried the *blaCTX* gene. However, 87.9% and 100% of the strains carried the *tetA* gene in the livestock slaughterhouse and urban sewages, respectively. In the current research, 46 (46.4%) strains were non-susceptible to nalidixic acid (Table 4). Moreover, 3 isolates which showed colistin resistance in the disk diffusion technique were meropenem susceptible.

According to the disk diffusion test (DDT) results, 21.2% of isolates were beta-lactamase producers (DDT-positive). Overall, 57.1% of DDT-positive strains were recovered from poultry sewage and 85.7% of DDT-positive isolates carried *fimH* virulence gene. Among 55 strains which revealed non-susceptibility to tetracycline, 53 (96.3%) samples carried *tetA* resistance trait in the PCR method. The data of the PCR demonstrated that 69.1% of cefuroxime non-susceptible strains carried *traT* and *fimH* surveyed virulence markers (Table 4).

Figure 1 displays the images of gel electrophoresis that was applied for the characterization of the amplified pathogenesis traits and non-susceptibility determinants in *E. coli* strains. The prevalence of *fimH*$^+$, *traT*$^+$, *blaCTX*$^+$, and *tetA*$^+$ genes was 91.9%, 89.9%, 79.8%, and 91.9%, respectively. Moreover, the *fimH* virulence marker was identified in isolates that were recovered from urban sewage with a frequency of 93.9% (n = 31). In the present study, the *blaCTX*$^+$ gene was the least gene that was identified among all strains (79.8%, n = 79). In ESBL-positive isolates, *traT* and *fimH* virulence markers were detected in 90.5% and 85.7% isolates, respectively (Table 4). Additionally, 76.19% and 90.5% of ESBL-positive isolates harboured *blaCTX* and *tetA* antibiotic resistance genes, respectively (Tables 4 and 5).

Among the studied isolates, *fimH*$^+$-*tetA*$^+$ genotype (84.84% strains) was the most common virulence marker

Table 4. Comparison of ESBL-Positive Isolates and Virulence and Antibiotic Non-susceptibility Markers Among *Escherichia coli* Strains Based on the Sample Type

		No. of isolates (%)	ESBL$^+$ (n=21, 21.2%)	ESBL$^+$, *blaCTX*$^+$ (n=15, 15.1%)	T_R, tetA$^+$ (n=53, 53.5%)	ESBL$^+$, *fimH*$^+$ (n=18, 18.8%)	ESBL$^+$, *traT*$^+$ (n=19, 19.1%)	*blaCTX*$^+$, *tetA*$^+$ (n=73, 73.7%)	*traT*$^+$, *tetA*$^+$ (n=82, 82.8%)	*fimH*$^+$, *tetA*$^+$ (n=84, 84.8%)	*blaCTX*$^+$, *traT*$^+$ (n=73, 73.7%)	*traT*$^+$, *fimH*$^+$ (n=81, 81.8%)
Specimen source	Poultry sewage	33	12 (57.1)	8 (53.3)	24 (45.3)	9 (50)	10 (52.6)	22 (30.1)	24 (29.3)	25 (29.8)	22 (30.1)	22 (27.2)
	Livestock slaughterhouse sewage	33	7 (33.3)	5 (33.3)	12 (22.6)	7 (38.9)	7 (36.8)	22 (30.1)	27 (32.9)	28 (33.3)	24 (32.9)	30 (37)
	Urban sewage	33	2 (9.5)	2 (13.3)	17 (32.1)	2 (11.1)	2 (10.5)	29 (39.7)	31 (37.8)	31 (36.9)	27 (37)	29 (35.8)
	Total	99 (100)	21 (100)	15 (100)	53 (100)	18 (100)	19 (100)	73 (100)	82 (100)	84 (100)	73 (100)	81 (100)

Note. ESBL$^+$: Extended spectrum beta-lactamase positive; T_R: Tetracycline resistant; *fimH*$^+$: *fimH* gene positive; *traT*$^+$: *traT* gene positive; *blaCTX*$^+$: *blaCTX* gene positive; *tetA*$^+$: *tetA* gene positive.

pattern, followed by *traT*$^+$-*tetA*$^+$ genotype (82.82% isolates), the details of which are provided in Table 5. Sixty-one (61.61%) out of 99 tested *E. coli* isolates showed *fimH*$^+$-*traT*$^+$-*blaCTX*$^+$-*tetA*$^+$ genotype (Table 5). Figure 1 depicts PCR data for the characterization of pathogenesis determinants and non-susceptibility traits in *E. coli* strains. In addition, 41% of isolates with *fimH*$^+$, *traT*$^+$, *blaCTX*$^+$, and *tetA*$^+$ genotype belonged to strains that were isolated from urban sewage.

Table 6 presents the comparison of non-susceptibility patterns, pathogenesis, and antibiotic resistance markers among the strains recovered from various sources. Among ESBL-positive isolates, 85.7% (n = 18) of them carried the *fimH* gene. In the current study, the highest rate of the *fimH* virulence gene was found in isolates recovered from livestock slaughterhouse sewage with a rate of 97% (n = 32). Further, the *traT* resistance gene was identified in 90.5% of ESBL-positive isolates. Overall, 19.8% of ESBL-positive strains carried *fimH* and *traT* virulence genes (*fimH*$^+$-*traT*$^+$ genotype).

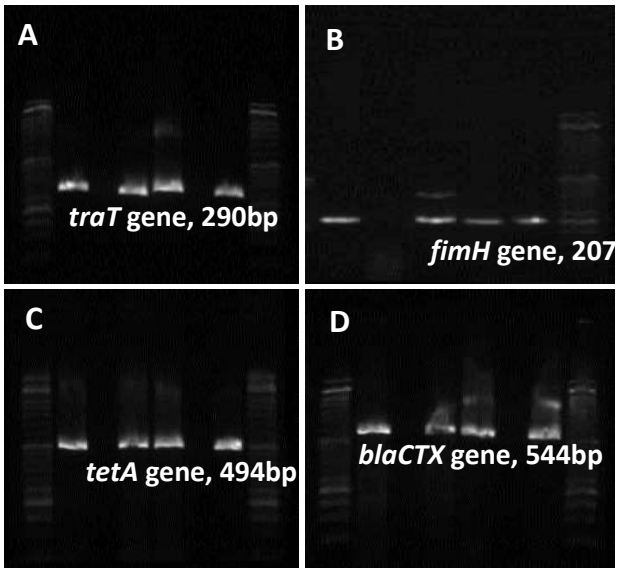

Figure 1. Agarose Gel Electrophoresis of the Amplified Virulence (*fimH* & *traT*) and Resistance Genes (*blaCTX* & *tetA*) by PCR Assay.
Note. **A.** *traT* gene (290 bp); **B.** *fimH* gene (207 bp); **C.** *tetA* gene (494 bp); **D.** *blaCTX* gene (544 bp). The 50 bp DNA ladder was applied in agarose gel electrophoresis.

Discussion

In the current cross-sectional research, a multicenter work was designed to assess the rate of multidrug resistance, antibiotic non-susceptibility patterns, beta-lactamase secretion, and virulence markers in *E. coli* recovered from wastewater samples in Gonabad. According to biochemical results, 99 *E. coli* samples were isolated from three different types of sewage (i.e., poultry, urban, livestock slaughterhouse sewages). Drug non-susceptibility in *E. coli* is a worldwide problem. The existence of various significant virulence markers and non-susceptibility to clinically related drugs, especially the emergence of MDR isolates, and their capability to deliver resistance markers to other microorganisms have made them a topic for research.

Penicillins, quinolones, cephalosporins, aminoglycosides, and antimetabolite are significant antibiotics in *E. coli* infection treatment. Moreover, it was vital to highlight the existence of 83 tigecycline-resistant, 67 cefuroxime-resistant, 46 nalidixic acid-resistant, 42 TM-resistant, and 3 colistin-resistant *E. coli* strains in the present work. In comparison with the same research conducted by Adefisoye et al (21), the resistance rate was higher in our study. Lately, it has been relatively prevalent to find non-

Table 5. Pathogenesis and Resistance Marker Patterns in *Escherichia coli* Strains

Profile	Virulence and Resistance Markers	Total 99 (100%)
A	*fimH*$^+$, *traT*$^+$, *blaCTX*$^+$, *tetA*$^+$	61 (61.6%)
B	*traT*$^+$, *blaCTX*$^+$, *tetA*$^+$	67 (67.6%)
C	*fimH*$^+$, *traT*$^+$, *tetA*$^+$	75 (75.7%)
D	*fimH*$^+$, *traT*$^+$, *blaCTX*$^+$	66 (66.6%)
E	*blaCTX*$^+$, *tetA*$^+$	73 (73.7%)
F	*traT*$^+$, *tetA*$^+$	82 (82.8%)
G	*blaCTX*$^+$, *fimH*$^+$	72 (72.7%)
H	*fimH*$^+$, *tetA*$^+$	84 (84.8%)
K	*traT*$^+$, *fimH*$^+$	81 (81.8%)
L	*blaCTX*$^+$, *traT*$^+$	73 (73.7%)

Table 6. Frequency of Studied Genes Among Strains Isolated From Different Sources and ESBL-Positive Isolates

		Virulence Genes (%)		Resistance Genes (%)	
		traT	*fimH*	*blaCTX*	*tetA*
Source (n=99)	Poultry sewage (n=33)	27 (81.8)	28 (84.8)	24 (72.7)	29 (87.9)
	Livestock slaughter house sewage (n=33)	31 (93.9)	32 (97)	26 (78.8)	29 (87.9)
	Urban sewage (n=33)	31 (93.9)	31 (93.9)	29 (87.9)	33 (100)
	Total	89 (89.9)	91 (91.9)	79 (79.8)	91 (91.9)
ESBL-positive strains (n=21)		19 (90.5)	18 (85.7)	16 (76.19)	19 (90.5)

Note. ESBL: Extended spectrum beta-lactamase positive.

susceptibility to colistin and carbapenems (22), which is in line with our findings. The long-term utilization of these drugs in community, hospitals, and veterinary workout in Iran can be the potential reason for the existence of colistin, carbapenem, nalidixic acid, and beta-lactam resistant isolates. Furthermore, ampicillin, trimethoprim, and trimethoprim-sulfamethoxazole resistance (22.2%, 42.4%, and 52.5%, respectively) was entirely high in the present study in comparison with data presented by Nerkar et al (23). The incidence of nalidixic acid resistant among poultry sewage isolates was 87.9% whereas that of the nalidixic acid resistant among urban sewage isolates was 18.2% in this study. The non-susceptibility profile of the strains for nitrofurantoin was lower (3%) in the current work, which corroborates with the findings (5.4% non-susceptible) of another investigation performed in the south Africa (21).

Depending on disk diffusion outputs, the results of the present study indicated that *E. coli* strains revealed a low prevalence of resistance to carbapenem (100% of isolates were susceptible to imipenem). This was in agreement with the results of other investigations, demonstrating the high rate of sensitivity to carbapenems among the wastewater isolates of *E. coli* (21, 24).

In the current research, 99% of surveyed isolates were found to be susceptible to ertapenem and isolates revealing similar susceptibility to imipenem and doripenem, but the rate of sensitivity to meropenem was higher (100%). The results further represented that the rate of carbapenem resistant was similar to another report on the prevalence of imipenem resistant *E. coli* (1%) in Vietnam (25). A similar enhancement in the frequency of carbapenem resistant *E. coli* was noticed in the study conducted in Japan (26) . The rapid rise in the rate of carbapenem non-susceptibility in Iran is worrying although carbapenems cannot serve as the antibiotic of choice for treating MDR-*E.coli* infections in the near future. The demonstrated level of nalidixic acid non-susceptibility (46.5%) among all surveyed strains was significantly higher than those reported formerly in similar investigations, which was generally about 31.4% (21). It should be also noted that in many cases of antibiotic resistance, bacterium is transmitted to the hospital environment and humans through other sources. As shown, the choice of drugs for the *E. coli* infection treatment in the hospitals is very restricted and some strains are non-susceptible to most common antibiotics.

Based on the DDT, the current investigation showed that 21.2% of *E. coli* isolates are ESBL-positive, and *blaCTX* gene was detected in 79.8% of isolates. Of all 21 ESBL-positive *E. coli* isolates, 15 (71.4%) samples carried the *blaCTX* marker while this marker was identified in 2 strains of colistin-resistant isolates. Based on the achieved data, the frequency of non-susceptibility among ESBL-positive isolates to nalidixic acid, ciprofloxacin, gentamicin, and nitrofurantoin was 71.4%, 28.6%, 0%, and 0%, respectively. This is consistent with the findings of a former study from Shanghai (China), which reported the presence of the *blaCTX* gene in 80% of the studied isolates (27).

In the current study, 3 strains were non-susceptible to colistin, which contradicts the results of a study in Nigeria, which reported that all strains were sensitive to colistin (28). The observed colistin non-susceptible strains in our research are quite alarming. Non-susceptible isolates were surveyed for alternative therapeutic items such as meropenem that revealed a good response to this drug (100% of the strains were susceptible). Colistin serves as a drug of last resort for MDR infections. In addition, the potential risk for colistin non-susceptible emerging in *E. coli* has significant clinical disorders in infection treatment guidelines.

The non-susceptibility to beta-lactams in *E. coli* is encoded by different genes such as the *blaCTX* marker. In this study, the frequency of *blaCTX* marker positive isolates was higher in the urban sewage (87.9%, n = 29) compared to the poultry sewage (72.7%, n = 24). Another alarming result was the relatively high frequency of cefuroxime non-susceptible *E. coli*. The rate of cefuroxime non-susceptible among the strains was found to be 67.6%. Further, the PCR results of the strains showed that 51 strains carried the *blaCTX* marker. These findings do not match with those of other studies conducted in Austria and India, demonstrating that the frequency of cefuroxime resistant was 11% and 39.1%, respectively (29,30). Considering the worldwide dissemination of nono-susceptibility to cephalosporins, as one of the most effective and accessible agents, powerful and active drugs such as the new generation of cephalosporins and carbapenems have been introduced during the past decade.

The resistance to tetracycline is encoded by the *tet* gene. In our research, 91.9% of isolates harboured the *tetA* gene.

Furthermore, the PCR data of strains confirmed that 53 tetracycline-resistant strains contained the *tetA* gene. All (100%, n = 33) urban sewage isolates harboured the *tetA* gene, In contrast, in the study by Adesoji et al, the presence of the *tetA* gene was reported in 55% of the strains (20).

The existence of virulence markers related to *E. coli* increased their virulence. Moreover, it was observed that urban sewage-recovered isolates had a higher number of pathogenesis markers in comparison with poultry sewage-recovered isolates. The findings of the current research demonstrated the high rate of *traT* and *fimH* virulence markers among *E. coli* strains, which is in line with the results of a previous study (31). Although the frequency of the *traT* gene was the same among hose and urban sewage isolates (93.9%), that of the *fimH* gene was not the same (97% vs. 93.9%). Our data are in agreement with those of another investigation from other geographical regions, showing the high frequency of the *fimH* virulence gene in *E. coli* and the high rate of the *blaCTX* marker among the strains (24,32). Additionally, *traT* and *fimH* genes were detected in 90.5%, and 85.7%, of the ESBL-positive *E. coli* strains, respectively, which corroborates with the findings of other researchers (33).

Our findings revealed that the frequency of resistant isolates is higher in poultry sewage strains compared to urban sewage strains. Totally, the presence of virulence genes was more prevalent in urban sewage strains in comparison with poultry sewage strains and the high frequency of multiple virulence markers could significantly contribute to the colonization and virulence of *E. coli* in the human community. In addition, the higher rate of *tetA* and *blaCTX* genes in poultry sewage strains may demonstrate the effect of broad antibiotic usage in the emergence of non-susceptibility to tetracyclines and beta-lactams.

Similarly, the *fimH* gene was identified in 91.9% of all investigated strains and livestock slaughterhouse sewage strains (n = 32, 97%) represented higher frequency as compared to urban (n = 31, 93.9%) and poultry (n = 28, 84.8%) sewages. This is in agreement with the results of another research, showing the *fimH* marker in about 86% of *E. coli* isolates (33). Accordingly, the *traT* marker was more common among ESBL-positive strains than the ESBL-negative ones. Likewise, they had a potential ability to present other pathogenesis markers and antibiotic non-susceptibility. Further, 61 (61.6%) isolates were identified as $fimH^+$, $traT^+$, $blaCTX^+$, and $tetA^+$ among which, only 13 (21.3%) samples were ESBL-positive. Furthermore, all ESBL-positive isolates were meropenem susceptible. These findings reveal that some of the isolates that carry the *blaCTX* marker are probably not identifiable by the DDT method or other contributing markers and mechanisms to non-susceptibility to beta-lactams. Based on the findings of the present study, the emergence of colistin and imipenem non-susceptible *E. coli,* principally in the human community, as well as the high rate of pathogenesis traits could be considered as a worrying situation. Drug non-susceptible pathogenic microorganisms can come from a variety of food sources (34-37).

Water organizes a good matrix for the lateral transmission of mobile genetic elements, which are regarded as the agents for the spread of pathogenesis or non-susceptibility determinants between the microorganisms from a variety of sources, participating in the change of natural bacterial ecosystems. Antibiotic resistance, particularly to beta-lactams, carbapenems, and colistin, is a potential threat for public health and the emergence of non-susceptible bacteria in environmental waters is an emerging problem worldwide.

In general, multiple non-susceptibility to the routinely applied drugs was high in the research area. This was even higher in hospital wastewater. The contamination of water by drugs or other pollutants leads to an increase in non-susceptibility because of selection pressure. Thus, an appropriate wastewater treatment plan should be considered and reclaimed and hygienic measures should be exercised as well. Our data confirmed the role of the sewage in the environmental transmission of virulent extra-intestinal pathogens, particularly ESBL-producing *E. coli*.

Conflict of Interests

Non to be declared.

Ethical Issues

The current study was carried out after the approval of the Ethics Committee of Gonabad University of Medical Sciences (code number IR.GUMS.REC.1396.74).

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